Sensitivity and Specificity of the Fluorescent Antibody Technique for Detection of Infectious Laryngotracheitis Virus

P. R. Ide*

ABSTRACT

The specificity of a fluorescent conjugate to infectious laryngotracheitis virus was examined using chick trachea organ culture or tissue sections infected with other avian viruses (adenovirus, infectious bronchitis, poxvirus, reovirus, Newcastle disease virus, Marek's disease virus, avian encephalomyelitis and infectious bursal agent) or Mycoplasma gallisepticum. Confirmation of virus replication in these preparations was obtained by either 1) demonstration of virus titre increase or 2) demonstration of fluorescence when using the homologous conjugate. Once either of these criteria had been satisfied, negative results with the infectious laryngotracheitis conjugate were taken to indicate that the conjugate would not present false positive results in differentiated cells infected with these heterologous viruses. The spectrum of reactivity of the infectious laryngotracheitis conjugate was then examined on organ cultures infected with several infectious laryngotracheitis isolates from across Canada. Finally, the conjugate was applied to experimental and natural cases of infectious laryngotracheitis and its efficiency was compared to routine virus isolation methods.

RÉSUMÉ

Cette expérience visait à vérifier la spécificité d'un conjugué fluorescent à l'endroit du virus de la laryngo-trachéite infectieuse, à l'aide de cultures de cellules de la trachée de poussins ou de coupes de tissus infectés avec les agents suivants: adénovirus, virus de la bronchite infectieuse, poxvirus, reovirus, virus de la pneumo-encéphalite aviaire, virus de la maladie de Marek, virus de l'encéphalomyélite aviaire, agent de la maladie des bourses de Fabricius, ou Mycoplasma gallisepticum. On confirma la réplication virale dans ces préparations en démontrant une augmentation 1) du titre des virus ou 2) de la fluorescence avec l'utilisation d'un conjugé homologue. Après avoir établi ces critères, on interpréta les résultats négatifs obtenus avec le conjugué spécifique au virus de la laryngo-trachéite infectieuse comme l'indice qu'il ne donnerait pas de faux résultats positifs dans les cellules différenciées et infectées avec les agents hétérologues. On vérifia ensuite l'éventail de réactivité de ce conjugué sur des cultures tissulaires infectées avec plusieurs souches du virus de la laryngo-trachéite infectieuse isolées un peu partout au Canada. On l'utilisa enfin dans des cas expérimentaux et naturels de laryngotrachéite infectieuse et on compara son efficacité avec celle des méthodes conventionnelles de l'isolation du virus.

INTRODUCTION

The fluorescent antibody (FA) technique has been applied to both experimental and natural cases of infectious laryngotracheitis (ILT) virus infection of chickens (1, 3, 11, 24). However, studies with experimental birds appear to have been conducted only with vaccine strains of ILT virus (2, 24) and reports on the application of the technique to field cases have been limited. For example, Braune (3) used FA for diagnosing seven field cases of ILT but details of the method were not given and

*Animal Pathology Division, Health of Animals Branch, Agriculture Canada, Atlantic Area Laboratory, P.O. Box 1410, Sackville, New Brunswick E0A 3C0.

Submitted February 24, 1977.

Mallinson (11) used the technique on an unspecified number of ILT field cases but no details of the test or the results were given. Furthermore, although it is usually considered that ILT isolates are of one serotype some evidence of serotype variation has been reported (4, 14) and studies to examine the spectrum of a given ILT conjugate against a variety of ILT isolates from different geographical areas have not been reported.

The following work was conducted to examine the specificity and diagnostic efficacy of a fluorescein conjugated ILT antiserum when used on 1) chick tracheal organ cultures or other differentiated tissues infected with various avian pathogens, 2) organ cultures infected with ILT isolates from widely separate geographical areas in Canada, 3) tracheas from birds experimentally infected with virulent or nonvirulent strains of ILT virus and 4) tracheas from chickens in field outbreaks of respiratory disease.

MATERIALS AND METHODS

ANIMALS

Eggs and experimental chickens were from an isolated nonvaccinated flock of Leghorn type birds which did not possess serum neutralizing antibody to the RV31 (see below) strain of ILT virus.

VIRUSES

The 18 ILT viruses used are designated by code and source as follows: RV31 (American Type Culture Collection, Lederle strain), V-73-20 (Dr. K. Lawson, Connaught Laboratories[1]), 8105 (Dr. A. Gagnon, OMAFF, Guelph, Ontario), V2337 and W1799 (Dr. N. Willis, A.D.R.I., Ottawa), local isolates SK27, 29, 30, 31, 32, 33, 35 and 36 and isolates from tracheas submitted from birds in British Columbia,[2] designated as ILT isolates 696, 721, 822, 1006 and 1113. Strain RV31 was cloned by three serial plaque passages in chick kidney (CK) tissue cultures and was used at a titre of $\log_{10}$ 5.1 EID_{50} (egg infectious dose 50 percent) per 0.2 ml. Other ILT isolates were not cloned prior to use. ILT strain V-73-20 which was selected for bird infectivity studies because of its field virulence was used at a titre of $\log_{10}$ 4.0 EID_{50}/0.2 ml.

Other (non-ILT) viruses and mycoplasma used were included in this work either because of their use in common avian vaccines or because of their potential as avian respiratory pathogens. These were avian adenovirus Indiana C and the Massachusetts (M41) and Connecticut strains of infectous bronchitis virus (Dr. R. W. Winterfield, Purdue University, Indiana), Newcastle disease virus strain B1 (American Type Culture Collection, Rockville, Maryland), avian reovirus strain Fahey-Crawley (Dr. N. Olson, University of West Virginia), an avian pox virus (Dr. N. Willis, A.D.R.I., Ottawa, isolate B5601) and *Mycoplasma gallisepticum* strain PG31 (NIAID, Bethesda, Maryland). Bursa of Fabricius tissue infected with Marek's disease (MD)[3] or infectious bursal agent (IBA) strain Sk-1 (9) and brain tissue infected with the Van Rockel strain of avian encephalomyelitis (AE) virus (8) were also used.

TISSUE CULTURES AND ORGAN CULTURES

Chick kidney (CK) tissue cultures were prepared as described by Churchill (5). Growth medium was Minimal Essential Medium (Eagle) containing Earle's salts without L-glutamine and sodium bicarbonate[4] to which was added, in final concentration, 0.5% lactalbumin hydrolysate, 5% tryptose phosphate broth,[5] 10% fetal calf serum (FCS) and antibiotics (100 i.u. penicillin, 100 mcg streptomycin, 25 units mycostatin[6] per ml) and 0.05% sodium bicarbonate. Maintenance medium was the same but contained 2% FCS. Cultures were maintained at 37°C in 5% CO2. No antibiotics were used on cultures infected with mycoplasma.

[1]Originally isolated at the Animal Pathology Pacific Area Laboratory from an outbreak of ILT in a flock at Abbotsford, B.C., January, 1973.

[2]Supplied by Dr. C. Cunningham, B.C. Provincial Veterinary Laboratory, Abbotsford, British Columbia.

[3]Tissue and conjugated antiserum to MD was supplied by Dr. L. Spencer, A.D.R.I., Ottawa, Ontario.

[4]Grand Island Biological Company, Grand Island, New York.

[5]Difco Laboratories, Detroit, Michigan.

[6]E. R. Squibb & Sons, New York.

Chick trachea organ cultures were prepared from two or three week old chicks as described by Darbyshire *et al* (7). Cultures were grown and maintained individually in tissue culture tubes using the medium described for CK cells but without FCS. Cultures were rolled at 37°C and were used on the third day after preparation when microscopic examination had confirmed ciliary activity.

After inoculation of 0.1 or 0.2 ml of virus suspension onto either monolayers or organ cultures from which growth medium was first removed, an adsorption period of one hour at 37°C was allowed before addition of maintenance medium. Cultures were examined using X30 magnification at daily intervals for up to seven days after inoculation.

Antiserum Production

Antiserum to ILT virus RV31 was produced in five month old chickens inoculated with 3 ml virus suspension intramuscularly at weekly intervals for six weeks. Sera collected before the first inoculation and three weeks after the last inoculation are referred to, respectively, as preinoculation serum and ILT (RV31) antiserum. Antiserum to another (undesignated) strain of ILT was obtained from a commercial source[7] and is designated as RS10 serum.

The plaque neutralization (80%) test for ILT (20) was conducted using serial twofold dilutions of serum, a virus challenge of approximately 80 plaque forming units (pfu) per 0.1 ml and a virus-serum incubation time of two hours at room temperature.

Fluorescent Antibody Methods

Fluorescein isothiocyanate conjugation of serum, conjugate titration, tissue sectioning and staining methods have been described (8, 9). Both preinoculation serum and ILT (RV31) antiserum were conjugated and are referred to as conjugated preinoculation serum and ILT conjugate, respectively. The specificity of positive fluorescence was determined as previously described (8) with the following modifications:

Antigen absorption test — The specificity of fluorescence induced by the ILT conjugate was checked by the antigen absorption test (13) using ILT virus RV31, ILT virus V-73-20 and control CK cells. Each virus infected CK pellet was prepared by freezing and thawing two infected 75 cm^2 surface area CK monolayers when virus cpe involved about 75% of the cells. This material (initial volume 60 ml) was sonicated[8] for ten sec and centrifuged at 2,500 g for 15 min. The supernatant was centrifuged at 35,000 g for two hr and the drained pellet was gently resuspended after soaking overnight at 4°C in 0.3 ml phosphate buffered saline (PBS) pH 7.2. Equal volumes of the resuspended pellet and ILT conjugate (at twice its normal working concentration) were mixed and incubated at 37°C for two hr. After centrifugation at 5,000 g for 15 min the supernatant was used as virus absorbed conjugate. Similar steps were taken using uninoculated CK cells to produce control-absorbed conjugate.

Heterologous agent fluorescence tests — These tests were conducted to determine whether the ILT conjugate would give positive results when used on differentiated cells infected with other avian pathogens. When conjugated antiserum to the non-ILT agents was available negative results with the ILT conjugate were accepted if, on duplicate tissue sections, positive results were obtained with the homologous (MD, IBD or AE or *Mycoplasma gallisepticum*) conjugate. However, when conjugated antisera to the non-ILT test viruses were not available additional steps were taken to determine that these viruses were replicating in the organ cultures. This was necessary to ensure that negative results were legitimately due to lack of cross reactivity of the ILT conjugate with the test viruses rather than lack of replication of the test viruses in the organ cultures. In this method five drained organ cultures were inoculated with 0.1 ml of test virus (IB, adeno, reo, pox or NDV) and after an adsorption period of one hr at room temperature, each was washed five times with 1 ml aliquots of maintenance medium to remove unadsorbed virus inoculum. A final 1 ml of maintenance medium was added to each culture but 0.1 ml was immediately removed and added to 0.9 ml PBS and frozen

[7]SPAFAS Incorporated, Norwich, Connecticut.

[8]Virsonic Cell Disrupter, Virtis Company, Gardiner, New York.

at –70°C for subsequent titration as the zero hr harvest. Cultures were incubated in roller drums at 37°C. At daily intervals for five days 0.1 ml of maintenance fluid from one organ culture was added to 0.9 ml PBS and this, plus cryostat sections prepared from the same culture were stored at –70°C. After all the organ cultures had been harvested in this manner, the stored fluids were titrated for infectivity by appropriate methods (in CK cultures for adenovirus, reovirus and NDV and in eggs for IB and pox viruses) and the sections were examined for fluorescence using ILT conjugate. A rising titre of virus in organ culture fluids indicated virus replication.

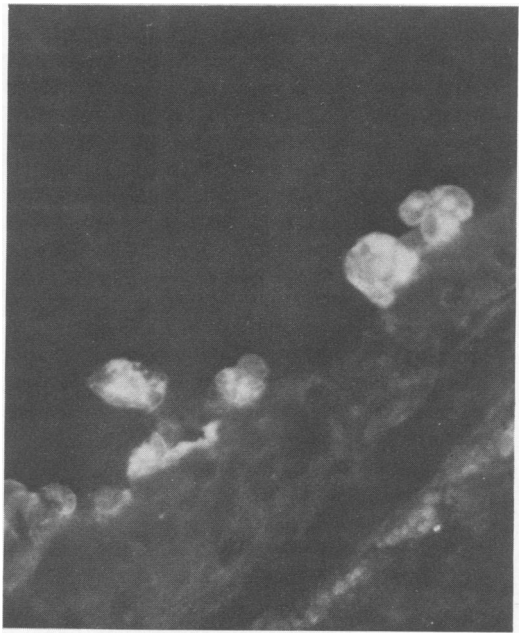

Fig. 2. Chick trachea organ culture 24 hr after inoculation with ILT strain RV31. Cryostat section stained with homologous fluorescein-conjugated antiserum. Fluorescence appears to be intranuclear in some of the cells.

Experimental and Natural Disease

Chickens between 16 and 22 weeks of age were inoculated with ILT strain V-73-20 or RV31 by deposition of 0.1 ml of inoculum into the anterior lumen of the trachea. Birds inoculated with each virus strain were housed in strict isolation.

Tissues from chickens in 16 commercial flocks (A to P) experiencing respiratory disease were examined. In the first nine flocks (A to I) ILT was suspected on the basis of clinical observation. Birds in the remaining flocks (J to P) exhibited only mild signs of upper respiratory disease and ILT was only peripherally considered in the clinical differential diagnosis. None of the flocks had been vaccinated against ILT.

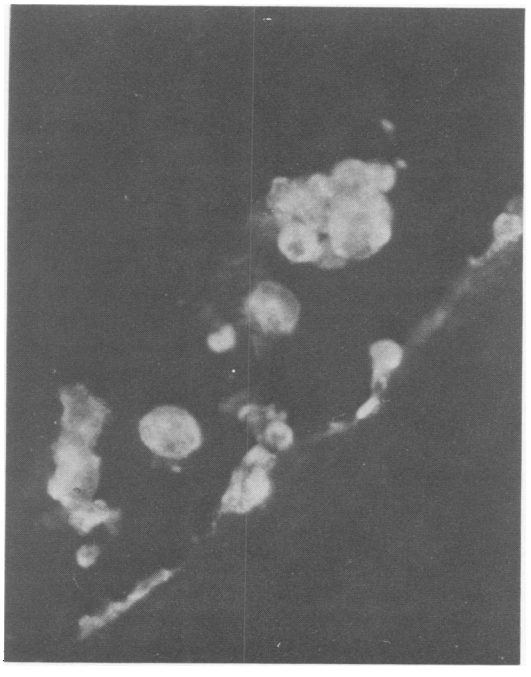

Fig. 1. Chick trachea organ culture 24 hr after inoculation with ILT strain RV31. Cryostat section stained with homologous fluorescein-conjugated antiserum. Note rounded cells, most of which are detached from the epithelial surface and fluorescence, which is primarily cytoplasmic.

Virus isolation from experimental birds was conducted by chorio-allantoic-membrane (CAM) inoculation of an approximate 10% homogenate of a 2 cm section of trachea in PBS containing 1000 i.u. penicillin, 1 mg streptomycin and 250 units mycostatin per ml according to standard methods (20). If no CAM lesions were observed when eggs were examined at postinoculation day (pid) 7, isolation was recorded as negative and no further passages were conducted. Virus isolations from field tissues were attempted by CAM and allantoic fluid (ALF) inoculation of an homogenate of an approximate 3 cm section of trachea and 1 cm^3 section of lung. In these cases isolation was considered negative only after three serial passages. Isolated viruses were identified by neutralization.

Histology

Organ cultures were fixed in Bouin's solution. Field specimens were fixed in formalin. Paraffin embedded sections were cut at 5 microns and stained with hematoxylin and eosin.

TABLE I. Titration of ILT in Chick Trachea Organ Cultures and Correlation Between Cytopathic Effect, Fluorescence and Infectivity Titre of Organ Culture Fluids

Log_{10} Dilution of Inoculum	Culture No.	cpe[a]	FA[b]	Titre of Fluids from Culture[c]
− 2	1	+	+	2.5
	2	+	+	3.0
	3	+	+	2.0
	4	+	+	2.5
− 3	5	+	+	3.5
	6	+	+	2.5
	7	+	+	3.0
	8	+	+	3.0
− 4	9	−	−	Neg.
	10	−	−	Neg.
	11	+	+	2.5
	12	−	−	Neg.
− 5	13	−	−	Neg.
	14	−	−	Neg.
	15	−	−	Neg.
	16	−	−	Neg.
Controls	17	−	−	Neg.
	18	−	−	Neg.
	19	−	−	Neg.
	20	−	−	Neg.

[a]Cytopathic effect in unstained cultures between pid 1 and 5
[b]Fluorescence of frozen sections stained with conjugated ILT antiserum
[c]Reciprocal of log_{10} $TCID_{50}$ in CK monolayer cultures

RESULTS

Serum

The homologous plaque neutralization (80%) titre of antiserum to ILT strain RV31 prior to conjugation was 1:2500. The working dilution of the ILT conjugate was 1:8. The plaque neutralization titre of serum RS10 against ILT strain RV31 was 1:128.

Organ Cultures

Tracheal organ cultures infected with ILT strain RV31 exhibited a cytopathic effect (cpe) characterized by epithelial cell enlargement and rounding, focal syncytial formation, cellular desquamation and patchy ciliostasis. Uninoculated cultures exposed to ILT conjugate exhibited no fluorescence but organ cultures infected with ILT strain RV31 exhibited bright fluorescence which was limited to epithelial cells. Affected cells, which were individual or in syncytia, were usually slightly or severely ballooned. Granular fluorescent material was seen primarily in the cytoplasm (Fig. 1) but in some cells concentrated fluorescence was also seen in the central and possibly nuclear area of the cell (Fig. 2).

Fluorescence was directly related to both the cpe and virus replication as monitored by titration in CK monolayer cultures (Table I).

Fluorescence Specificity

Preliminary tests — No fluorescence was seen when ILT-infected organ cultures

TABLE II. Fluorescent Antibody Trials on Chick Trachea Organ Cultures Infected with Various Viruses when Homologous Conjugated Antisera were not Available

	Day Harvested							
	0		2		3		5	
	a	b	a	b	a	b	a	b
Infectious bronchitis (Massachusetts)	0.2		2.5	−	3.0	−		
Infectious bronchitis (Connecticut)	0.5		1.2	−	1.2	−		
Newcastle disease virus	3.0		4.0	−	4.0	−		
Adenovirus	0.5		1.5	−	2.0	−		
Reovirus	0.5		3.0	−	3.5	−		
Poxivirus	1.0				2.75	−	3.0	−
ILT strain V-73-20 (positive control)	0.5		2.5	+	3.0	+		

a = Infectivity titre ($TCID_{50}$/0.2 ml) of organ culture fluids on the day harvested
b = Positive (+) or negative (−) FA results using ILT conjugate
Blank = not done

TABLE III. Comparison of Fluorescent Antibody and CAM Isolation Methods for Detection of ILT Virus Isolates RV31 and V-73-20 in Experimental Chickens

	Infected With							
	V-73-20				RV31			
Pid Killed	Bird	FA	Isolation	Clinical	Bird	FA	Isolation	Clinical
1 - 2.....	1	+	+	N	1	−	−	N
	2	−	−	N	2	−	−	N
	3	−	−	N	3	−	+	N
	4	−	−	N	4	−	+	N
3 - 5.....	5	+	+	D	5	−	+	MRD
	6	+	+	D	6	+	+	MRD
	7	+	+	D	7	−	−	MRD
	8	+	+	SRD	8	+	+	MRD
	9	+	+	SRD	9	+	+	MRD
	10	−	+	SRD				
	11	+	+	SRD				
7.....	12	−	−	N	10	−	−	N
	13	−	−	N	11	−	−	N
	14	−	−	N				
14.....	15	−	−	N	12	−	−	N
	16	−	−	N	13	−	−	N
	17	−	−	N				

N = Clinically normal, D = Died, SRD = Severe respiratory disease, MRD = Mild respiratory disease

were stained with conjugated preinoculation serum. The fluorescence of positive cultures was reduced by 80-100% when cultures were exposed to unconjugated ILT (RV31) antiserum prior to exposure to ILT conjugate (homologous serum blocking test) but was not reduced when similarly pretreated with unconjugated preinoculation serum. However, blocking was only at a level of 10 or 20% when ILT antiserum (RS10) from a commercial source was used in place of antiserum to strain RV31. When the ILT conjugate was adsorbed with either RV31 or ILT strain V-73-20 the ability of the conjugate to produce fluorescence on cultures infected with RV31 was reduced by 90-100% (homologous and heterologous ILT absorption test). No reduction of fluorescence was seen when the ILT conjugate was absorbed with the uninoculated control pellet of CK cells.

Heterologous reactivity tests — Organ cultures infected with infectious bronchitis viruses (Connecticut and Massachusetts strains), Newcastle disease virus, reovirus, adenovirus and an avian pox virus did not exhibit fluorescence when stained with the ILT conjugate despite the fact that virus replication, evidenced by a rising virus titre, was demonstrated in the cultures (Table II). Furthermore, frozen sections of AE-infected chick brain, IBA or MD-infected chick bursa and *M. gallisepticum*-infected trachea organ cultures did not fluoresce after exposure to the ILT conjugate. Strong fluorescence was seen when duplicate sections were stained with homologous conjugated antiserum to the respective viruses.

Homologous reactivity spectrum — Tracheal organ cultures inoculated with 17 ILT virus isolates from widely separate geographical areas in Canada (British Columbia, Ontario, Prince Edward Island and Nova Scotia) all gave positive fluorescence when examined using the ILT conjugate (antiserum to ILT strain RV31). Both the cytopathic effect and the fluorescence observed were the same as that described for strain RV31. When duplicate sections of the positive cultures were stained with ILT conjugate previously absorbed with ILT strain RV31 or when cultures were first exposed to unconjugated homologous ILT antiserum, fluorescence was reduced by 90-100%.

Comparison of FA and Isolation Methods in Experimental and Natural Disease

Experimental disease — Results are presented in Table III. In birds inoculated

with the virulent ILT field strain V-73-20 deaths and severe clinical signs of respiratory disease occurred on pid 3 to 5, but recovery was rapid in surviving birds and apart from very mild residual signs of tracheitis all were clinically normal on pid 7. Post mortem lesions of hemorrhagic tracheitis were seen on pid 3-5. By pid 7 the tracheal epithelium was macroscopically normal but there was a slightly increased amount of mucus in the lumen. In birds inoculated with the avirulent ILT strain (RV31) only very minor signs of upper respiratory disease were seen in a few birds on pid 4 and 5 and no macroscopic evidence of tracheitis was seen. The correlation between positive FA and isolation results was good but in some cases, particularly in birds infected with RV31, isolation was positive when FA was negative. This was particularly evident in birds without clinical disease.

Natural disease— In studies on 21 birds from nine ILT-positive flocks (A to I) the correlation between FA and ILT virus isolation was good, with the exception of one bird in which isolation was positive when FA was negative. In most cases FA results were definitive and in all cases positive fluorescence was significantly (80-100%) blocked by unconjugated ILT (RV31) antiserum.

Histological tracheitis was observed in all these birds but epithelial intranuclear inclusions were not seen in the tracheas of birds from three flocks despite the fact that the same tracheas had given positive FA and virus isolation results. Conversely, intranuclear inclusions were seen in the tracheal epithelium of one bird which gave negative FA and isolation results.

With one exception ILT virus was isolated on the first CAM passage. In this case, which required three serial passages before CAM lesions were seen, fluorescence of the trachea had been very weak and poorly defined.

Fluorescent antibody examination of tracheas from 15 birds from the remaining seven flocks (J to P) using the ILT conjugate gave negative results. Isolation attempts from four of these were unsuccessful but infectious bronchitis virus was isolated from two flocks and *Mycoplasma* spp. were isolated from another flock.

DISCUSSION

These results indicated that the FA technique could be applied to the diagnosis of natural ILT infection to provide a rapid and accurate means of virus identification in tracheal epithelial cells. The correlation between FA and virus isolation was good in birds experimentally infected with a virulent ILT strain (Table III) and in natural cases of the disease. This correlation was not as good in birds experimentally infected with a mild strain of ILT (Table III).

For individual birds within these three groups (birds experimentally infected with a mild strain of ILT, a virulent strain of ILT or naturally infected birds) virus isolation was sometimes positive when FA was negative. This was particularly evident in birds infected with the mild strain of ILT, indicating that in routine diagnostic tests negative FA should be followed by isolation attempts.

Several of the tracheas from field cases examined were removed from birds that were dead (within 24 hrs) when submitted to the laboratory, but virus isolation and FA results compared favorably with results on submitted live birds. Published evidence has indicated that storage of tracheas for longer than 48 hrs (at 4°C) prior to FA examination increased the possibility of false negative results (2). As a result and also because strong positive results were obtained with tracheas from birds killed *in extremis* (Table III), it is recommended that the FA method be conducted, when possible, only on birds killed when showing severe signs of upper respiratory disease and that FA examination of tracheal tissue be conducted as soon as possible after death. Within these parameters the FA method for ILT diagnosis compared favorably in accuracy and speed with other methods. For example, virus isolation may take between five and 14 days to complete, gel diffusion may take from 24 hrs to four days to complete (10) and electron microscopy which may be less sensitive than virus isolation (15, 16) is not readily available to many diagnostic laboratories. In this work, birds from three of the field outbreaks did not possess demonstrable intranuclear inclusion bodies in tracheal epithelial cells whereas FA and isolation results confirmed the diagnosis of ILT. Similar results were reported by Van

der Heide *et al* (24).

The location of fluorescence in ILT infected cells was primarily cytoplasmic, although some apparent intranuclear fluorescence was seen in a few cells. Considerable variation has been reported in descriptions of the location of ILT fluorescence. For example, Van der Heide *et al* (24) observed both intranuclear and intracytoplasmic fluorescence in the tracheal epithelial cells of chicks infected with a vaccine strain of ILT. Sharma *et al* (18) described intranuclear fluorescence followed by intracytoplasmic fluorescence in ILT-infected tissue culture cells but Mayer *et al* (12) described only intranuclear fluorescence in infected chick kidney cells. Conversely, Reynolds *et al* (17) observed only cytoplasmic fluorescence in ILT-infected chick embryo kidney monolayers and suggested that this would be expected if antibody against ILT had been prepared using the intact enveloped virion as antigen. Although one report suggested the presence of intranuclear enveloped ILT particles (15) it has been demonstrated that the ILT virion assumes an envelope and its characteristic appearance and size only after emerging from the nucleus (21, 22, 23). It is possible that different descriptions of the FA reaction in ILT-infected cells are simply indications of the relative proportions of enveloped and nonenveloped virus particles in the antigen used for immunization.

Positive results were obtained when the conjugated ILT antiserum was tested on organ cultures infected with ILT isolates from across Canada. Such results were not entirely unexpected, as *in vitro* serological comparisons of ILT iolates have usually indicated serotype homology (6, 19) but some *in vitro* and *in vivo* serotype variation has been reported among ILT isolates (4, 14) and even among other apparently homologous isolates *in vivo* protection tests and *in vitro* neutralization tests have given different results (19). However, results presented here support a conclusion that the spectrum of fluorescent reactivity of conjugated antiserum to the RV31 (Lederle) strain of ILT was of sufficient width to be of diagnostic use.

In studies to determine the specificity of the FA reaction both the homologous ILT-serum blocking test and the homologous and heterologous ILT-strain absorption test indicated that the fluorescence was specific for ILT virus antigen. The heterologous ILT-serum blocking test using ILT antiserum from another laboratory was not successful. This may have been due to a difference in avidity between the homologous and heterologous ILT sera, with replacement of the unconjugated ("blocking") serum by conjugated serum (13) and the difference in the ILT neutralizing titre between the "blocking" serum (1:128) and serum subsequently conjugated (1:2500) may have affected these results. Another interpretation of this failure to block would have been that the fluorescent reaction was not specific for ILT. However, this was disproved by the homologous and heterologous ILT strain absorption tests and by results with experimental and natural cases of ILT infection (which were confirmed by virus isolation and identification procedures).

Negative results obtained when the ILT conjugate was used on organ cultures infected with other avian pathogens further supported the conclusion that the homologous reaction was specific for ILT. As the degree of cross fluorescence between agents within each group (IB, ND, adenovirus, poxvirus, reovirus, AE, IBA, MD and *M. gallisepticum*) is not known, results of these tests cannot be extrapolated to infer that the ILT conjugate would not possibly react with other members of these virus groups but they do indicate that such false positive reactions would not be expected.

ACKNOWLEDGMENTS

The capable technical assistance of Mr. M. Briscoe, Mr. D. Rayworth and Mrs. L. Griffin is gratefully acknowledged. Thanks are extended to my colleagues Dr. W. F. DeWitt for providing clinical data and field submissions, and Dr. R. G. Stevenson for histological studies of field cases.

REFERENCES

1. **BRAUNE, M. O. and R. F. GENTRY.** Detection of avian respiratory viruses in experimental birds and tissue culture by the F.A. technique. Proceedings 37th Northeastern Conference on Avian Diseases, Pennsylvania State University. June 21-23, 1965.
2. **BRAUNE, M. O. and R. F. GENTRY.** Standardization of the fluorescent antibody technique for the detection of avian respiratory viruses. Avian Dis. 9: 535-545. 1965.
3. **BRAUNE, M. O.** Diagnosis by fluorescent antibody. Results of 253 suspected respiratory cases. Proceedings 37th Northeastern Conference on Avian Diseases, Pennsylvania State University, June 21-23, 1965.

4. CHRISTOVA, Z. and V. N. SYURIN. Variants of the virus of avian infectious laryngotracheitis. Abstracted in Vet. Bull. 34: 374b. 1964.
5. CHURCHILL, A. E. The use of chicken kidney tissue culture in the study of the avian viruses of Newcastle disease, infectious laryngotracheitis and infectious bronchitis. Res. vet. Sci. 6: 162-169. 1965.
6. COVER, M. S. and W. J. BENTON. The biological variation of the infectious laryngotracheitis virus. Avian Dis. 2: 378-383. 1958.
7. DARBYSHIRE, J. H., J. K. COOK and R. W. PETERS. Comparative growth kinetic studies on avian infectious bronchitis virus in different systems. J. comp. Path. 85: 623-630. 1975.
8. IDE, P. R. Application of the fluorescent antibody technique to the diagnosis of avian encephalomyelitis. Can. J. comp. Med. 38: 49-55. 1974.
9. IDE, P. R. A comparison of gel diffusion fluorescent antibody and virus isolation methods in experimental and natural cases of infectious bursal disease. Can. J. comp. Med. 39: 183-190. 1975.
10. JORDAN, F. T. and R. C. CHUBB. The agar gel diffusion technique in the diagnosis of infectious laryngotracheitis (ILT) and its differentiation from fowl pox. Res. vet. Sci. 3: 245-255. 1962.
11. MALLINSON, E. T. A diagnostician's impression of the F.A. test in the routine diagnosis of avian viral respiratory disease. Proceedings 37th Northeastern Conference on Avian Diseases, Pennsylvania State University. June 21-23, 1965.
12. MAYER, V. M., F. M. HETRICK, W. D. KEENUM and H. M. DEVOLT. Replication of infectious laryngotracheitis virus in chicken kidney cell culture. Am. J. vet. Res. 28: 825-832. 1967.
13. NAIRN, R. C. Fluorescent Protein Tracing. pp. 131-143. London: E and S Livingstone Ltd. 1964.
14. PULSFORD, M. H. Possible P.Q. type variation in infectious laryngotracheitis. Nature, Lond. 172: 1193-1195. 1953.
15. PURCELL, D. A. The ultrastructural changes produced by infectious laryngotracheitis virus in tracheal epithelium of the fowl. Res. vet. Sci. 12: 455-458. 1971.
16. PURCELL, D. A. and J. B. McFERRAN. Influence of method of infection on the pathogenesis of infectious laryngotracheitis. J. comp. Path. 79: 285-291. 1969.
17. REYNOLDS, H. A., A. M. WATRACH and L. E. HANSON. Development of the nuclear inclusion bodies of infectious laryngotracheitis. Avian Dis. 12: 332-347. 1968.
18. SHARMA, J. M., L. G. RAGGI and F. GRAMENZI. Pathogenesis of infectious laryngotrachetitis virus in chicken kidney cells. Am. J. vet. Res. 30: 1843-1850. 1969.
19. SHIBLEY, G. P., R. E. LUGINBUHL and C. F. HELMBOLDT. A study of infectious laryngotracheitis virus. I. Comparison of serologic and immunogenic properties. Avian Dis. 6: 59-71. 1962.
20. TRIPATHY, D. N. and L. E. HANSON. Laryngotracheitis. In Isolation and Identification of Avian Pathogens. American Association of Avian Pathologists. pp. 227-234. Ithaca, New York: Arnold Printing Corp. 1975.
21. WATRACH, A. M. Intranuclear filaments associated with infectious laryngotracheitis virus. Virology 18: 324-327. 1962.
22. WATRACH, A. M., L. E. HANSON and M. A. WATRACH. Crystalline aggregates of infectious laryngotracheitis virus. Virology 35: 321-323. 1968.
23. WATRACH, A. M., A. E. VATTER, L. E. HANSON, M. A. WATRACH and H. E. RHOADES. Electron microscopic studies of the virus of avian infectious laryngotracheitis. Am. J. vet. Res. 20: 537-544. 1959.
24. VAN DER HEIDE, L., H. L. CHUTE and D. C. O'MEARA. A comparative investigation of the course of an experimental infection with infectious laryngotracheitis in chickens. Avian Dis. 11: 149-153. 1967.

BOOK REVIEW

RECENT ADVANCES IN MEDICAL AND VETERINARY MYCOLOGY. *Edited by Kazuo Iwata. Published by University Park Press, Baltimore. 1977. 316 pages. Price $60.00.*

Medical mycology came of age during the fifties and early sixties as a result of the activities of a number of mycologists and medical clinicians who brought order into the taxonomy of the pathogenic fungi and laid down procedures for the diagnosis and treatment of mycoses. Current research in the field is presented in this book which draws together three special contributions and thirty-seven papers presented at the 6th Congress of Human and Animal Mycology held in Tokyo in 1975. The papers are grouped in six sections and range over the current status of immunology and of therapy of the mycoses, recent advances in the mechanisms of fungal infections, the problems due to opportunistic fungi, the ecology and epidemiology of animal mycoses and the taxonomy of the pathogenic fungi.

The book opens with an urbane review of milestones in the history of medical mycology by Dr. Ajello. This is followed by papers dealing with the immunological response, both cellular and antibody, to fungi infections, and the importance of serological tests in the recognition and monitoring of these diseases. A welcome list of fungal antigens maintained at the Mycological Reference Laboratory in London, England is included. The mechanisms which enable some fungi to overcome the host's responses and to adapt to a pahogenic life style are discussed by a number of authors including Dr. Iwata, whose closing lecture on the role of fungal toxins in the etiopathology of fungal infections is printed in full. The section on therapy of the mycoses is mainly concerned with the evaluation of 5-fluorocytosine in the treatment of yeast and other infections. Mycotic infections in animals, mostly dermatophytic, are described from various parts of the world, and several authors comment on the increasing incidence of animal mycoses due to modern farming methods with resulting production losses and public health problems.

This book is not intended for veterinary practitioners but will be a valuable reference work for everyone engaged in research and teaching in the field of medical and veterinary mycology. — *W. M. Dion.*